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Transmission and evolution of bacteria during the course of enteritis outbreaks

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Abstract

Bacterial enteritis outbreaks are a worldwide problem. They are hard to investigate as the bacterial agents are often associated with multiple sources, closely-related bacteria often co-colonise these sources, highly discriminatory tests are often required to distinguish between these bacteria, and bacteria are continuously evolving, changing how they behave. In this thesis I investigated the transmission and evolution of bacteria over the course of enteritis outbreaks by integrating genomic, phenotypic and antibiotic susceptibility testing, and phylogenetic modelling in four studies.

The aim of the first study was to investigate the origin, evolution and transmission of *Salmonella enterica* serovar Typhimurium DT160 over a 14-year long outbreak in New Zealand. Genomic analysis of 109 DT160 isolates collected over this timeframe established that the DT160 strain was introduced into New Zealand approximately a year before the first human isolate was reported; there were frequent transmissions between the source groups investigated (human, wild bird, poultry and bovine); and there was no evidence of specific selective pressures imposed on DT160. This study demonstrated how genomic analyses can be used to investigate extended outbreaks of bacterial diseases.

The aim of the second study was to investigate whether two ancestral state reconstruction models (the discrete trait analysis and structured coalescent models) were applicable to salmonellosis outbreak investigations. Both models were used to estimate transmission and population parameters of simulated salmonellosis outbreaks. Comparisons between the models' estimates and the true transmission and population values for the simulations revealed that both models made assumptions that did not apply to outbreaks and prevented them from accurately predicting these parameters. This study highlighted the need for outbreak-specific phylogenetic transmission models.

The aim of the third study was to investigate the relationship between two strains of *Salmonella* that were the predominant causes of human salmonellosis in New Zealand in the 2000s (*S. Typhimurium* DT160 and *S. Typhimurium* DT56 variant), and identify potential reasons for one strain declining (DT160) as the other emerged (DT56 variant). This study demonstrated how genomic analyses can be used to compare *Salmonella* strains and identify genetic elements that may influence strain behaviour.

The aim of the fourth study was to investigate a patient that had presented excreting the same genotype of *Campylobacter*, *C. jejuni* ST45, on multiple occasions over a 10-year period. Genomic analyses, phenotypic testing and antimicrobial susceptibility testing of sixteen *Campylobacter* isolates collected from the

patient found that the patient was persistently colonised with *Campylobacter* over this period, and that the *Campylobacter* had adapted to long-term colonisation by altering its motility and developing resistance to the antibiotics the patient had been prescribed. This study demonstrated how genomic analyses can be used to investigate a patient's infection history.

These studies demonstrated the applicability and limitations of genomic analyses when investigating bacterial enteritis outbreaks, how genetics and the environment influence bacterial evolution, and highlighted areas in the fields of microbiology, phylogenetics, epidemiology and public health that require further research.

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Abbreviations

μ l	Microlitre
°C	Degrees Celsius
×g	Times gravity
bp	Base pairs
CDC	Centre for Disease Control and Prevention
<i>C. difficile</i>	<i>Clostridium difficile</i>
CE	Common Era
<i>C. jejuni</i>	<i>Campylobacter jejuni</i>
CI	Confidence interval
COG	Cluster of Orthologous Group
CRC	Canterbury Regional Council
CVID	Common variable immune deficiency
Df	Degrees of freedom
DNA	Deoxyribonucleic acid
DT	Definitive type
DTA	Discrete trait analysis
EFSA	European Food Safety Authority
<i>E. coli</i>	<i>Escherichia coli</i>
ENA	European Nucleotide Archive
ESR	Institute of Environmental Science and Research

EUCAST	European Society of Clinical Microbiology and Infectious Diseases
GC	Guanine-cytosine
GMRF	Gaussian Markov Random Fields
GTR	Generalised time reversible
GWRC	Greater Wellington Regional Council
HBRC	Hawke's Bay Regional Council
HKY	Hasegawa, Kishino and Yano
HPD	Highest posterior density
HIV	Human immunodeficiency virus
IgA	Immunoglobulin A
IgG	Immunoglobulin G
INDELS	INsertions/DEletions
IVABS	Institute of Veterinary, Animal and Biological Sciences
kb	Kilo base pairs
Mb	Mega base pairs
MCDHB	MidCentral District Health Board
MCMC	Markov chain Monte Carlo
MDS	Multidimensional scaling
^mEpiLab	Massey University Molecular Epidemiology and Public Health Laboratory
ml	Millilitre
mm	Millimetre
mM	Millimolar
MLST	Multilocus sequence typing
MSE	Mean squared error
MSS	Mean sum of squares
MSSS	Manawatu sentinel surveillance site

NCBI	National Centre for Biotechnology Information
NZGL	New Zealand Genomics Limited
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PNMRF	Palmerston North Medical Research Fund
SC	Structured coalescent
<i>S. enterica</i>	<i>Salmonella enterica</i>
<i>S. Enteritidis</i>	<i>Salmonella enterica</i> serovar Enteritidis
SIR	Susceptible-Infected-Recovered
SNP	Single nucleotide polymorphism
SS	Sum of squares
ST	Sequence type
STEC	Shiga toxin-producing <i>Escherichia coli</i>
<i>S. Typhi</i>	<i>Salmonella enterica</i> serovar Typhi
<i>S. Typhimurium</i>	<i>Salmonella enterica</i> serovar Typhimurium
UK	United Kingdom
US	United States
WHO	World Health Organisation
XML	Extensible Markup Language

Publications

Journals

Bloomfield, S.J., Benschop, J., Biggs, P.J., Marshall, J.C., Hayman, D.T.S., Carter, P.E., Midwinter, A.C., Mather, A.E. and French, N.P. (2017) Genomic analysis of *Salmonella enterica* serovar Typhimurium DT160 associated with a 14-year outbreak, New Zealand, 1998-2012. *Emerging Infectious Diseases* 23: 906-913

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